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Viable Endospores of *Thermoactinomyces vulgaris* in Lake Sediments as Indicators of Agricultural History

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Bacteria of the genus *Thermoactinomyces* form endospores with an extreme longevity in natural habitats. We isolated *Thermoactinomyces sacchari* from 9,000-year-old varved (annually laminated) sediment; thus, *T. sacchari* is probably one of the oldest known living organisms. More importantly, we tested and verified the hypothesis that there is a relationship between concentrations of dormant, viable endospores of *T. vulgaris* in lake sediments and the extent of agriculture in the catchments of the lakes. In surface sediments, low concentrations were recorded in forest lakes and the concentrations increased with increasing areas of cultivated land around the lakes. In varved sediment cores from three lakes, we found a temporal relationship between records of *T. vulgaris* endospores and the pollen of plants indicating agriculture. Endospores were very rare in sediments deposited before agriculture, ca. 1100 A.D. From then to between 1300 and 1700 A.D., a period with restricted cultivation, low but more regular rates of accumulation of endospores were recorded. High endospore accumulation rates were found with the subsequent agricultural expansion. This investigation confirms suggestions that this bacterium could be used as a paleoindicator for agricultural activity and be complementary to pollen analyses. Viable bacteria in continuous records of lake sediments are also potential material for evolutionary studies.

Bacteria of the genus *Thermoactinomyces* are very common in agricultural environments. They grow, for example, in compost, rotten hay, and manure (1). *Thermoactinomyces* spp. form very resistant endospores (4). Lake sediments have been used to study the longevity of these organisms (2), and several-thousand-year-old living endospores have been recovered (10, 14, 17). Their value for paleoecological studies has also been indicated. In the English Lake District, high concentrations of *Thermoactinomyces vulgaris* endospores were recorded in surface sediment samples from lakes surrounded by cultivated land (3). In Seamere (East Anglia), a core section (ca. 1950 to 2750 before present [B.P.]) in which the percentages of certain kinds of tree pollen declined and in which cereal and weed pollen appeared contained viable thermoactinomycete endospores (17). Viable endospores have also been found in occupational debris from ca. 100 A.D. at archaeological excavations of Fort Vindolanda (14). These studies have led to the suggestion that this bacterium could be useful in paleoecological studies (17).

We tested the hypothesis that there is a relationship between the number of *T. vulgaris* endospores deposited in lake sediments and the extent of agriculture in the lake catchments. If there is such a relationship, analyses of *T. vulgaris* contents in lake sediment cores could be used to study the history of agriculture. Bacterial analyses could become a quick complementary method for pollen analyses, which are very time-consuming.

MATERIALS AND METHODS

This investigation comprised two approaches: (i) analyses of surface sediment samples from forest lakes and lakes with agriculture in their catchments and (ii) analyses of sediment cores from three lakes with known agricultural histories determined from pollen analyses. A few samples of forest and pasture soils were also analyzed.

Lake surface samples (0 to 1 cm) were taken with a modified Kajak corer, and samples were placed in plastic bags immediately in the field. All sediment samples and cores were taken through ice in winter, when spore concentrations in the air are expected to be negligible. Soil samples were taken with a steel tube (diameter, 24 mm) which was pushed down through the organic horizon in the forest soil and through the ploughed horizon in pasture soil. Each soil sample consisted of amalgamated material from 10 subsamples.

Sediment cores were taken with a Russian corer from three lakes in Northern Sweden (Judesjön, 62°50′ N, 17°42′ E; Koltjärn, 62°57′ N, 18°18′ E; and Kassjön, 63°55′ N, 20°01′ E). These lakes have varved sediments, i.e., unmixed sediments with clearly distinguishable annual layers (12). Chronologies were obtained by varve counting (13). Unconsolidated surface sediments are not retained by the Russian corer: therefore, sediments from the last decades were not analyzed.

Cores and samples were kept in a cold room and analyzed within 24 h. Surface sediment and soil samples were mixed in their plastic bags and subsampled with a sterile spoon. Sediment cores were carefully cleaned by repeated cross-scraping (along the varves) with a sterile stainless steel knife. After cleaning of the core surfaces, samples of about 0.5 g were taken from the interior of the cores and placed in tubes with sterile Winogradsky solution (11). Sediment aggregates were crushed with a glass rod, and the suspension was mixed with a shaker (30 s, 20 Hz). It is important to standardize the mixing and the subsampling of the suspension because the endospores are probably aggregated to various sediment particles. Five to 10 replicate samples were spread on petri dishes with a selective medium containing novobiocin, and the plates were incubated at 50°C for 4 days (3). This selective medium excludes the growth of both *Bacillus* spp.

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and other thermophilic actinomycetes (1). The isolates were identified to the species level by growth conditions, colony morphology, and microscopic characters. *T. vulgaris* grows quickly and forms flat colonies with a white aerial mycelium, while *T. sacchari* grows slowly and forms colorless colonies with no visible aerial mycelium (9). Net annual rates of accumulation of *T. vulgaris* endospores were calculated by using endospore concentrations per gram of fresh sediment, sediment water content, and sediment accumulation rates obtained from varve thickness measurements.

A number of experiments were done to prove that the results obtained were not caused by contamination. These comprised a comparison of the endospore concentrations in pairs of samples taken along the uncleaned surface of a core and from the interior; if the corer transported endospores from the sediment-water interface down into older layers, a higher *T. vulgaris* concentration would be expected on the core surface. A test of the sediment core subsampling procedure described above was made by smearing a core with a suspension of *Penicillium* spores. About 5×10^4 spores cm^{-2} were added where subsampling from the interior of the core was done. A similar test was also done with tritium-labeled *Escherichia coli*.

Pollen analysis is the standard technique for studying agricultural history and has been applied in numerous studies (5). The quantitative pollen analyses presented here were made by U. Segerström and J.-E. Wallin by using contiguous core samples and a slightly modified standard method (15).

RESULTS AND DISCUSSION

Undisturbed varves in the cores guaranteed that no mixing of the sediments had occurred after sediment deposition in the lakes or during coring. No significant differences in endospore concentrations were found in the pairs of samples from the core surface and core interior. No *Penicillium* spores or radioactivity were recovered in the samples from the interior of the core. These tests indicated that the results were not influenced by contamination, i.e., that endospores from the surface sediment were not transported down by the corer.

T. vulgaris requires temperatures of 30 to 60°C and aerobic conditions (1) and cannot grow in anoxic sediments in lake bottoms, with temperatures rarely above 4°C. All these data support the conclusion that the isolated bacteria were from dormant, viable endospores incorporated into the sediments as these were deposited in the lakes. During a period of 5 years, *T. vulgaris* has been analyzed from several cores and sets of subsamples from Koltjärn and Kassjön by slightly modified methods; similar results have always been obtained, indicating the reliability of the method used here.

We recorded low concentrations of *T. vulgaris* endospores in the forest soil samples, as well as low concentrations in surface sediments from forest lakes as compared with lakes in agricultural areas (Table 1). Also, there was reasonable agreement between the number of endospores in lake surface sediments and the area of cultivated land in the catchments (Table 1). This result confirms that this species is rare in natural boreal forest ecosystems but very common in agricultural systems.

In all three lake cores, we found a clear temporal relationship between *T. vulgaris* endospores and agricultural pollen indicators. With the start of agriculture, endospore accumulation rates increased from sporadic to more regular values, and later agricultural expansion was accompanied by a further increase (Fig. 1). In Koltjärn, peaks of both *T. vulgaris* and cultural pollen at about 500 A.D. suggest Iron Age culture near Koltjärn. There was a significant statistical relationship between mean annual rates of accumulation of endospores ($\log_{10}$) and of cultural pollen. The trend explained 83% of the variance for Kassjön, 67% for Koltjärn, and 58% for Judesjön (t test, $P < 0.001$ in all cases). When this trend was subtracted, regression of the endospore residuals on pollen accumulation rates explained an additional 8% ($P < 0.01$) of the original variance in Kassjön and 12% ($P < 0.001$) in Koltjärn.

TABLE 1. Recovered viable endospores of *T. vulgaris* in soil samples and in surface sediment samples (0 to 1 cm) from lakes in forests and agricultural areas ($n = 3$)

Material and location	No. of spores g^{-1} (dry wt)	SD
Soil samples		
Pine forest (organic horizon)	0	
Spruce forest (organic horizon)[a]	100	100
Pasture, grazed (ploughed horizon)	9,200	1,200
Pasture, abandoned (ploughed horizon)	28,000	9,400
Sediments from forest lakes		
Abborrtjärn (Norrbotten)	200	200
Abborrtjärn (Västerbotten)	1,200	
Långtjärn	2,200	1,000
Stinttjärn	400	200
Sediments from lakes with agricultural catchments (cultivated area, in ha)		
Västerbackatjärn (20)	19,000	2,600
Judesjön (50)	78,000	15,000
Tavelsjöavan (240)	168,000	92,000
Kassjön (300)	308,000	3,500

[a] About 500 m from pastures.

Analyses of sediments deposited in Kassjön from 1000 to 5000 B.P. were also done. This was a period with no agriculture, according to the pollen analyses. Only sporadic colonies of *T. vulgaris* were found, indicating low natural background concentrations. Viable spores of *T. sacchari*, another species found in these sediments, were recovered from 9,000-year-old varved sediment from another lake (Sarsjön, 64°02′ N, 19°36′ E); this was the oldest sediment available. Therefore, this bacterium is one of the oldest known living organisms (6–8, 16).

The temporal correlation between the pollen and spore curves (Fig. 1) and early endospore accumulation rate maxima in Koltjärn (Iron Age, ca. 500 A.D.) and Judesjön (1600 to 1800 A.D.) confirmed that the results were not die-off curves, although increased mortality with time is a factor that must be taken into account. However, on the basis of experiments with *Bacillus subtilis*, another endospore-forming bacterium, it has been argued that the duration of viability of endospores might be on the order of 200,000 years or longer (7).

Both the core studies and the analyses of surface sediments support the hypothesis that this bacterium could be used as a paleoindicator for agricultural activity in boreal ecosystems. However, in Elk Lake in Minnesota, Parduhn and Watterson (10) found high numbers of *T. vulgaris* endospores in 7,000-year-old sediments which they suggested were caused by dry and warm conditions and expansion of a semiarid prairie (10). This result indicates that natural vegetation changes in grass-rich ecosystems may also be important factors determining endospore records in lake sediments.

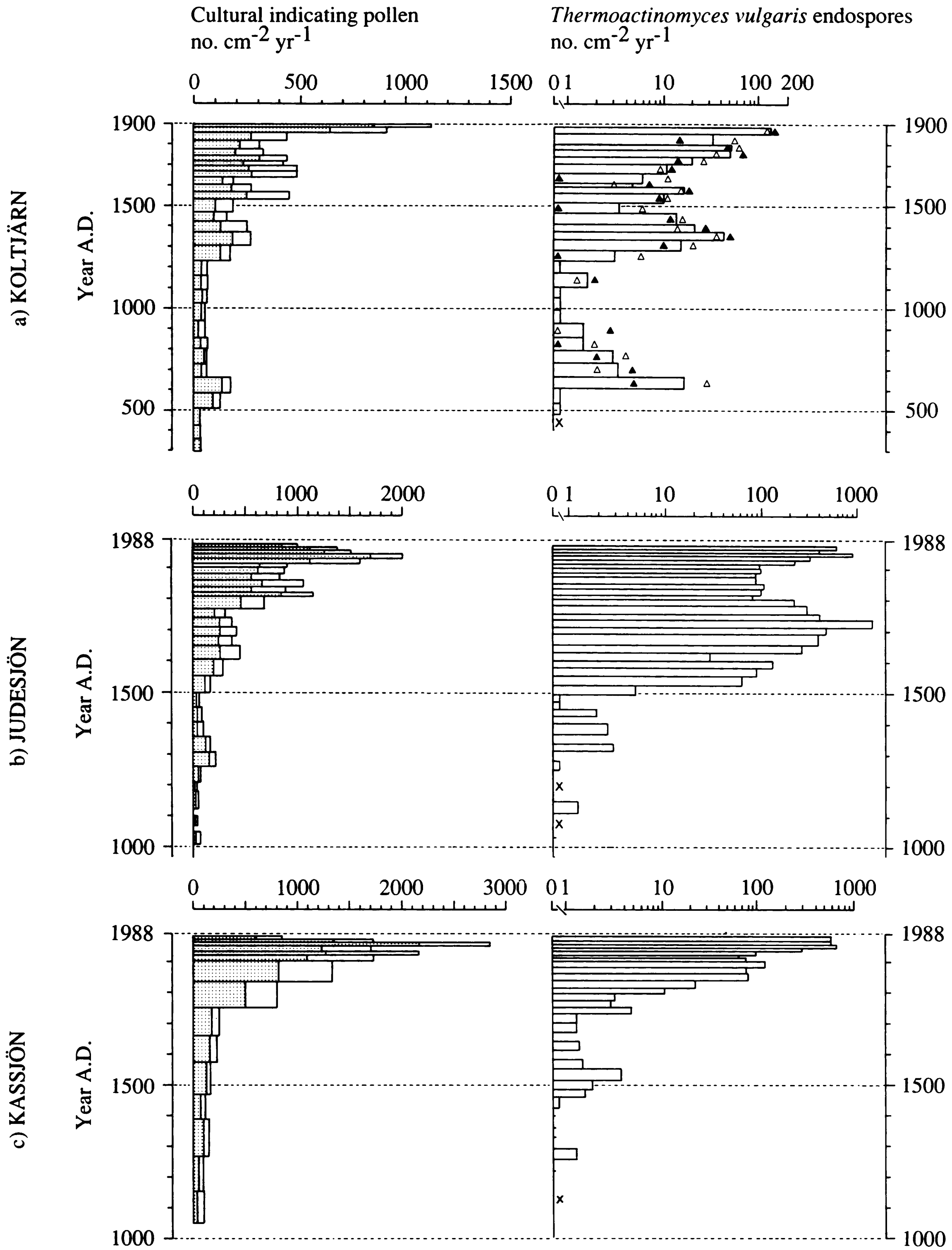

FIG. 1. Net annual rates of accumulation of pollen indicating agriculture (left panels) and viable endospores of *T. vulgaris* (right panels) in the sediments of lakes Koltjärn (a), Judesjön (b), and Kassjön (c) (x, not analyzed). In the pollen histograms, shaded areas indicate grass and open areas indicate other cultural indicators, such as cereals and weeds. In the *T. vulgaris*-Koltjärn histogram, mean values of analyses of two sets of samples (△ and ▲) from the same core (log scale) are shown. The variation was generally rather small; some variation was probably caused by the facts that each sample covered 20 to 50 years and the technique used did not allow sampling of exactly the same amount of sediment from each individual year. Analyses of consecutive samples, each covering just a few years, suggested short-term temporal variation in the net accumulation of endospores of at least the same order as the sampling variation obtained from the duplicate analyses of the Koltjärn core presented here.

In our study, the pollen and endospore profiles differed slightly in some details; e.g., the *T. vulgaris* peak from 1600 to 1800 A.D. in Judesjön had no pollen equivalent. Such discrepancies may have resulted because endospores and pollen reflect different agricultural practices and conditions (fields, pasture, abandoned land, cattle, etc). If that is the case, *T. vulgaris* records could provide alternative and additional information about agricultural history to supplement that derived by pollen analysis. Importantly, bacterial analysis of a sediment core can be made in a fraction of the time that pollen analysis takes.

There are a number of other nonsediment bacteria (e.g., *Bacillus* and *Clostridium* spp.) that have the ability to survive in lake sediments. Besides having paleoecological applications, all these organisms could have potential value in evolutionary studies.

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